targetingsystems

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Targeting Systems Introduces :

Targefect-Neuronal

Suggested transfection protocol for Neuronal cells

Cells incubated with transfection complexes in the absence of serum

Please vortex F-1 reagent at full speed for 30 seconds once or twice just before use. Do not vortex the F-2 reagent or Virofect

Conditions recommended most are in bold.

Protocol 1

Transfection of cells in presence of serum :

Step1 : Preparation of transfection complexes - Please prepare transfection complexes using serum free DMEM. Use 2 ml or 5 ml sterile clear polystyrene tubes. Make additions as follows :

Tube #	High Glucose DMEM	DNA	Targefect	Virofect
1	0.5 ml	6 µg	12 µl F-1	25 µl
2	0.5 ml	6 µg	12 µl F-2	25 µl
3	0.5 ml	6 µg	12 µl F-1	
4	0.5 ml	6 µg	12 µl F-2	
5	0.5 ml	10 µg	20 µl F-2	

• Add DMEM to tubes, first add DNA, mix well, then add targefect, mix well, add enhancer last and mix well again. Incubate at 37º C to form transfection complexes. Add 70 µl of transfection complex to 0.5 ml of complete media per well of a 24 well dish
• Add 125 µl of transfection complex to 0.5 ml of complete media (with 10% serum) per well of a 12 well dish
• Add 250 µl of transfection complex to 1ml of complete media per well of a 6 well dish.

Swirl the dish to mix transfection complexes with complete media. Replace media with fresh culture media the next day. Assay at 30-48 hrs post transfection.

Protocol 2 - Transfection of cells at the time of plating

Complex 3 µg DNA with 12 µl F-1 and 12.5 µl of Virofect in 0.25 ml DMEM

Incubate at 37 °C for 25 mins to form complexes

Add 0.25 ml of transfection complex to 2 ml of complete media per well of a 6-well dish immediately after plating cells or

If transfecting in 24-well dishes, add 0.05 ml complex to 0.5 ml of complete media in one well of a 24 well dish.

Complex 3 µg DNA with 12 µl F-1 and 25 µl of virofect in 0.25 ml DMEM

Incubate at 37 °C for 25 mins to form complexes

Add 0.25 ml of transfection complex to 2 ml of complete media per well of a 6-well dish immediately after plating cells or

Add 0.05 ml complex to 0.5 ml of complete media in one well of a 24 well dish.

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Assay at 36-48 hrs post transfection
These last two conditions give very high efficiencies in other difficult to transfect cells.

Protocol 3: Transfection in the absence of serum

Prepare transfection complexes as follows :

Tube #	High Glucose DMEM	DNA	Targefect	Enhancer reagent
1	1 ml	1.5 µg	5 µl F-2	-
2	1 ml	2 µg	5 µl F-1	-
3	1 ml	5 µg	5 µl F-1	5 µl Peptide enhancer
4	1 ml	2 µg	5 µl F-1	5 µl Virofect
5	1 ml	2 µg	6 µl F-2	5 µl Virofect

Add DMEM to tubes, first add DNA, mix well, then add targefect, mix well, Add enhancer last and mix well again . Incubate at 37 °C to form transfection complexes.

Add 1ml of transfection complex per well of a 6-well dish, Add 0.3 ml of transfection complex per well of a 12-well dish or 0.15 ml of transfection complex per well of a 24 well dish (make sure cells are covered well or else add 0.2 ml complex per well. Incubate cells with transfection complexes at 37 °C for 3 hrs. Aspirate transfection complexes and add complete media with serum.

Add 0.5 ml complete media per well of a 24 well dish, 1 ml per well of a 12-well dish, 2 ml per well of a 6-well dish. Mix gently and incubate overnight. Replace media the next day. Assay at 30-48hrs post transfection. We recommend using media with 10% serum as the complete media